09/664186
A# 9

WEST Search History

DATE: Friday, May 24, 2002

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L10	l1 with L9	61	L10
L9	plasmid	46671	L9
L8	l1 with L7	0	L8
L7	bifunctional or dual	293236	L7
L6	l4 or l5	7	L6
L5	l1 with L3	1	L5
L4	l1 with l2	7	L4
L3	rept	921	L3
L2	shuttle	35269	L2
L1	thermus	1963	L1

END OF SEARCH HISTORY

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☐ 1. 6350591. 16 Feb 99; 26 Feb 02. Recombinant DNA and methods for producing thermostable enzymes. Weber; J. Mark, et al. 435/69.1; 435/477 536/23.7. C12N015/74 C12N015/31.

☐ 2. 6344327. 12 Apr 00; 05 Feb 02. Methods for isolation of thermophile promoters. Peredultchuk; Mikhail, et al. 435/6; 435/252.3 435/29 435/440 435/471 435/477 435/69.1 536/23.1 536/24.1. C12Q001/68 C12Q001/02 C12N001/20 C12N015/00 C07H021/04.

☐ 3. 6294358. 07 Sep 99; 25 Sep 01. Thermus promoters for gene expression. Peredultchuk; Mikhail, et al. 435/69.1; 435/252.3 435/320.1 435/440 435/477 435/6 536/23.1 536/24.1. C12P021/00 C12N015/00 C12N015/74 C07H021/04.

☐ 4. 6207377. 14 Aug 98; 27 Mar 01. Method for construction of thermus-E. coli shuttle vectors and identification of two Thermus plasmid replication origins. Wayne; Jay, et al. 435/6; 435/252.3 435/320.1 435/471 435/91.1 536/23.1 536/24.1. C12Q001/68 C12P019/34 C12N015/74 C12N015/63 C12N001/20.

☐ 5. 5872238. 18 Aug 97; 16 Feb 99. Thermophile gene transfer. Weber; J. Mark, et al. 536/23.7;. C12N015/31.

☐ 6. 5786174. 28 Jan 97; 28 Jul 98. Thermophile gene transfer. Weber; J. Mark, et al. 435/69.1; 435/463 530/350 536/23.1. C12P021/02 C12N015/63 C07K014/00 C07H021/04.

☐ 7. 5120658. 28 Mar 89; 09 Jun 92. Thermostable tryptophan synthetase gene and extremely thermophilic plasmid vector incorporating said gene. Koyama; Yoshinori, et al. 435/320.1; 435/108 435/183 435/252.3 435/69.1 435/71.2 435/91.41 536/23.2. C12N015/70 C12N015/52.

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☐ 2. 20020025517. 09 Nov 98. 28 Feb 02. METHODS AND COMPOSITIONS FOR CELLULAR AND METABOLIC ENGINEERING. MINSHULL, JEREMY, et al. 435/6; 435/91.2 C12Q001/68 C12P019/34.

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☐ 4. 6352847. 08 Jun 99; 05 Mar 02. Ammonia elimination liquid reagent. Matsukawa; Hirokazu, et al. 435/190; 424/94.4 435/26. C12N009/04 C12Q001/32 A61K038/44.

☐ 5. 6350591. 16 Feb 99; 26 Feb 02. Recombinant DNA and methods for producing thermostable enzymes. Weber; J. Mark, et al. 435/69.1; 435/477 536/23.7. C12N015/74 C12N015/31.

☐ 6. 6344327. 12 Apr 00; 05 Feb 02. Methods for isolation of thermophile promoters. Peredultchuk; Mikhail, et al. 435/6; 435/252.3 435/29 435/440 435/471 435/477 435/69.1 536/23.1 536/24.1. C12Q001/68 C12Q001/02 C12N001/20 C12N015/00 C07H021/04.

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☐ 11. 6287863. 09 Nov 95; 11 Sep 01. Method of transferring a DNA sequence to a cell in vitro. Hodgson; Clague P.. 435/455; 435/400 435/456 514/44 800/19 800/23. C12N015/85 C12N015/00.

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1. Document ID: US 6350591 B1

L6: Entry 1 of 7

File: USPT

Feb 26, 2002

US-PAT-NO: 6350591
DOCUMENT-IDENTIFIER: US 6350591 B1

TITLE: Recombinant DNA and methods for producing thermostable enzymes

DATE-ISSUED: February 26, 2002

US-CL-CURRENT: 435/69.1; 435/477, 536/23.7

APPL-NO: 9/ 250585
DATE FILED: February 16, 1999

PARENT-CASE:

This application is a continuation of application Ser. No. 08/912,794, filed Aug. 18, 1997, now U.S. Pat. No. 5,872,238, which is a continuation of application Ser. No. 08/496,932 filed Jun. 30, 1995, now abandoned, which is a continuation of application Ser. No. 08/265,522, filed Jun. 24, 1994, now abandoned.

IN: Weber; J. Mark, Demirjian; David C., Casadaban; Malcolm J., Vonstein; Veronika, Pagratis; Nikos C.

AB: We have developed a new gene transfer system for extreme thermophiles of the genus Thermus, including Thermus flavus., using a chromosomal gene, and a thermostable derivative of the kanamycin-resistance gene (kan.sup.tr2). A plasmid mediated gene-replacement process is used to insert it into the chromosome resulting in the production of Leu.sup.- Km.sup.r transformants. This system not only allows stable, single-copy gene insertion into the chromosome of an extreme thermophile, but can be used in the thermo-genetic process described here to generate thermo-stabilized enzymes and proteins for industrial processes. This host-vector environment makes it possible to generate further thermo-stabilizing mutations in the kan gene beyond those levels previously reported.

L6: Entry 1 of 7

File: USPT

Feb 26, 2002

DOCUMENT-IDENTIFIER: US 6350591 B1
TITLE: Recombinant DNA and methods for producing thermostable enzymes

Brief Summary Paragraph Right (9):
Koyama et al. (1990) A plasmid vector for an extreme thermophile, Thermus thermophilus, FEMS Microbiology Letters 72:97-102, teach a Thermus-E. coli shuttle vector carrying a tryptophan synthetase gene (trpB). This cryptic plasmid pTT8, was able to transform Thermus thermophilus. The authors point out that a plasmid vector carrying trpBA was not suitable for selection since the cloned DNA fragment recombined with the chromosomal counterpart at high frequency.

Brief Summary Paragraph Right (17):
Lasa et al. (1992a) Development of Thermus-Escherichia Shuttle Vectors and Their Use for Expression of the Clostridium thermocellum celA Gene in Thermus thermophilus, J. of Bacteriology 174:6424-6431, teach the self-selection of undescribed origins of replication from cryptic plasmids from uncharacterized Thermus spp. and Thermus aquaticus are isolated and cloned into E. coli vectors. Plasmids were constructed with these origins, pLU1 to pLU4 from T. aquaticus, and pMY1 to pMY3 from Thermus spp. The plasmids then had a modified form of the cellulase gene (celA) from Clostridium thermocellum and were expressed in E. coli with the signal peptide from the S-layer gene from T. thermophilus. Transformation back into T. thermophilus allowed for expression at 70.degree. C.

2. Document ID: US 6344327 B1

L6: Entry 2 of 7

File: USPT

Feb 5, 2002

US-PAT-NO: 6344327
DOCUMENT-IDENTIFIER: US 6344327 B1

TITLE: Methods for isolation of thermophile promoters

DATE-ISSUED: February 5, 2002

US-CL-CURRENT: 435/6; 435/252.3, 435/29, 435/440, 435/471, 435/477, 435/69.1, 536/23.1, 536/24.1

APPL-NO: 9/ 548260
DATE FILED: April 12, 2000

PARENT-CASE:

This application is a divisional application of U.S. patent application Ser. No. 09/390,867, filed Sep. 7, 1999.

IN: Peredultchuk; Mikhail, Vonstein; Veronica, Demirjian; David

AB: The present invention relates to a system for identifying, isolating and utilizing promoter elements useful for expression of nucleotide sequences and the proteins encoded thereby in a thermophile. In one embodiment, a recombinant DNA molecule is provided, and comprises a reporter sequence, a putative thermophile promoter, a selectable marker sequence, and a 3' and a 5' DNA targeting sequence that are together capable of causing integration of at least a portion of said DNA molecule into the genome of a thermophile. Further, within the recombinant DNA, the reporter sequence is under the transcriptional control of a promoter which functions in a thermophile to form a promoter/reporter cassette, the promote/reporter cassette is flanked by said 3' and said 5' DNA targeting sequences, and the promoter/reporter cassette is positioned in the opposite orientation of the DNA targeting sequences.

L6: Entry 2 of 7

File: USPT

Feb 5, 2002

DOCUMENT-IDENTIFIER: US 6344327 B1
TITLE: Methods for isolation of thermophile promoters

Drawing Description Paragraph Right (3):
FIG. 3. Construction of pTG200 and development of promoter test vectors. A) Comparison of terminator sequences from Thermus. The his terminator was used in the construction of pTG200. B) pTG200 consists of an E. coli shuttle vector with the Thermus leuB gene disrupted by the promoterless kantr2 gene in the opposite direction. A strong Thermus transcription terminator is placed ownstream of the Kantr2 gene to prevent transcription through the gene in the opposite

direction. Promoter-test vectors were constructed by using primers to the two ends of the kan gene with an extended 50-60 bp promoter attached at the 5' end. Leu terminator (SEQ ID NO. 47); his terminator (SEQ ID NO. 48); icd terminator (SEQ ID NO. 49); proC terminator (SEQ ID NO. 50); phe S/T terminator (SEQ ID NO. 51); pol terminator (SEQ ID NO. 52).

Detailed Description Paragraph Right (5):
Using the reagents and techniques described in this application, inducible and constitutive promoters, integrative and plasmid-based vectors, and nucleic acids containing secretion signals may be isolated. The vectors utilized may be any vector suitable to isolation and characterization of a promoter. For instance, the vectors utilized may be plasmid, bacteriphage, virus, phagemid, cointegrate of one or more species, etc. Preferably, the vector is amenable to expression of a nucleotide sequence in a prokaryotic cell such as Thermus or E. coli. It is further preferable that the vectors be capable of functioning in different types of cells (ie, shuttle), such as Thermus or E coli.

Detailed Description Paragraph Right (10):
Liao, et al. ((1986) Isolation of a thermostable enzyme variant by cloning and selection in a thermophile. Proc. Natl. Acad. Sci. USA. 83:576-580) first demonstrated in vivo thermostabilization of a gene by using kanamycin nucleotidyl transferase in Bacillus stearothermophilus where resistance to 63.degree. C. was shown. To improve the genetic thermostabilization approach, a gene transfer system for Thermus was developed where the upper growth limit was above 80.degree. C. instead of 65.degree. C. as in Bacillus (described in, for example, U.S. Pat. No. 5,786,174 which is hereby incorporated by reference). These experiments were intitially conducted using the thermostabilized kan gene, in which the initial Km.sup.r supported growth only to 55.degree. C. in Thermus and not to 63.degree. C. as reported by Liao, et. al. ((1986) Isolation of a thermostable enzyme variant by cloning and selection in a thermophile. Proc. Natl. Acad. Sci. USA 83:576-580). The regulated expression system provided herein allows for fine-tuning of thermostabilization selection experiments so that the temperature range can be regulated and controlled and cutoff temperatures for selection adjusted in subsequent rounds of mutagenesis. Some important elements of Thermus' genetic background have been previously described. The generation of mutations (Koyama, et al. (1990) Cloning and sequence analysis of tryptophan synthetase genes of an extreme thermophile, Thermus thermophilus HB27: Plasmid transfer from replica-plated Escherichia coli recombinant colonies to competent T. thermophilus cells. J. Bacteriol. 172:3490-3495; Koyama, et al. (1990) A plasmid vector for an extreme thermophile, Thermus thermophilus. FEMS Microbiol. Lett. 72:97-102; Lasa, et al. (1992) Insertional mutagenesis in the extreme thermophilic eubacteria Thermus thermophilus HB8. Molec. Microbiol. 6:1555-1564), chromosomal integration (Koyama, et al. (1990) Cloning and sequence analysis of tryptophan synthetase genes of an extreme thermophile, Thermus thermophilus HB27: Plasmid transfer from replica-plated Escherichia coli recombinant colonies to competent T. thermophilus cells. J. Bacteriol. 172:3490-3495; Koyama, et al. (1990) A plasmid vector for an extreme thermophile, Thermus thermophilus. FEMS Microbiol Lett. 72:97-102; Lasa, et al. (1992) Insertional mutagenesis in the extreme thermophilic eubacteria Thermus thermophilus HB8. Molec. Microbiol. 6:1555-1564), plasmids (Mather, et al. (1990) Plasmid-associated aggregation in Thermus thermophilus HB8. Plasmid. 24:45-56; Hishinuma, et al. (1978) Isolation of extrachromosomasl deoxyribonucleic Acids from extremely thermophilic bacteria. Jour. of General Microbiology. 104:193-199.). and phages (Sakaki, et al. (1975) Isolation and Characterization of a Bacteriophage Infectious to an Extreme Thermophile. Thermus thermophilus HB8. J. Virol. 15:1449-1453) have also been studied. Several successful attempts to develop cloning systems using plasmids and chromosomal integration systems were demonstrated (Koyama, et al. (1986) Genetic transformation of the extreme thermophile thermos thermophilus and of other thermos spp. J. Bacteriol. 166:338-340; Lasa, et al. (1992) Development of Thermus-Escherichia Shuttle Vectors and Their Use for Expression of the Clostridium thermocellum celA Gene in Thermus thermophilus. J. Bacteriol. 174:6424-6431; Mather, et al. (1992) Development of Plasmid Cloning Vectors for Thermus thermophilus HB8: Expression of a Heterologous, Plasmid-Borne Kanamycin Nucleotidyltransferase Gene. Appl. Environ. Microbiol 58:421-425.). However, none of these provide the versatility as those provided herein.

Other Reference Publication (14):
Lasa et al., "Development of Thermus-Escherichia Shuttle Vectors and Their Use for Expression of the Clostridium thermocellum celA Gene in Thermus thermophilus," J. of Bacteriology, 174:6424-6431 (1992a).

3. Document ID: US 6294358 B1

L6: Entry 3 of 7 File: USPT

Sep 25, 2001

US-PAT-NO: 6294358
DOCUMENT-IDENTIFIER: US 6294358 B1

TITLE: Thermus promoters for gene expression

DATE-ISSUED: September 25, 2001

US-CL-CURRENT: 435/69.1; 435/252.3, 435/320.1, 435/440, 435/477, 435/6, 536/23.1, 536/24.1

APPL-NO: 9/ 390867
DATE FILED: September 7, 1999
IN: Peredultchuk; Mikhail, Vonstein; Veronica, Demirjian; David C.

AB: The present invention relates to a system for identifying, isolating and utilizing promoter elements useful for expression of nucleotide sequences and the proteins encoded thereby in a thermophile. In one embodiment, a recombinant DNA molecule is provided, and comprises a reporter sequence, a putative thermophile promoter, a selectable marker sequence, and a 3' and a 5' DNA targeting sequence that are together capable of causing integration of at least a portion of said DNA molecule into the genome of a thermophile. Further, within the recombinant DNA, the reporter sequence is under the transcriptional control of a promoter which functions in a thermophile to form a promoter/reporter cassette, the promote/reporter cassette is flanked by said 3' and said 5' DNA targeting sequences, and the promoter/reporter cassette is positioned in the opposite orientation of the DNA targeting sequences.

L6: Entry 3 of 7 File: USPT

Sep 25, 2001

DOCUMENT-IDENTIFIER: US 6294358 B1
TITLE: Thermus promoters for gene expression

Drawing Description Paragraph Right (3):
FIG. 3. Construction of pTG200 and development of promoter test vectors. A) Comparison of terminator sequences from Thermus SEQ ID NO: 47-SEQ ID NO: 52. The his terminator was used in the construction of pTG200. B) pTG200 consists of an E. coli shuttle vector with the Thermus leuB gene disrupted by the promoterless kantr2 gene in the opposite direction. A strong Thermus transcription terminator is placed downstream of the kantr2 gene to prevent transcription through the gene in the opposite direction. Promoter-test vectors were constructed by using primers to the two ends of the kan gene with an extended 50-60 bp promoter

attached at the 5'end.

Detailed Description Paragraph Right (5):
Using the reagents and techniques described in this application, inducible and constitutive promoters, integrative and plasmid-based vectors, and nucleic acids containing secretion signals may be isolated. The vectors utilized may be any vector suitable to isolation and characterization of a promoter. For instance, the vectors utilized may be plasmid, bacteriophage, virus, phagemid, cointegrate of one or more species, etc. Preferably, the vector is amenable to expression of a nucleotide sequence in a prokaryotic cell such as Thermus or E. coli. It is further preferable that the vectors be capable of functioning in different types of cells (ie, shuttle), such as Thermus or E. coli.

Detailed Description Paragraph Right (10):
Liao, et al. ((1986) Isolation of a thermostable enzyme variant by cloning and selection in a thermophile. Proc. Natl. Acad. Sci. USA. 83:576-580) first demonstrated in vivo thermostabilization of a gene by using kanamycin nucleotidyl transferase in Bacillus stearothermophilus where resistance to 63.degree. C. was shown. To improve the genetic thermostabilization approach, a gene transfer system for Thermus was developed where the upper growth limit was above 80.degree. C. instead of 65.degree. C. as in Bacillus (described in, for example, U.S. Pat. No. 5,786,174 which is hereby incorporated by reference). These experiments were intitially conducted using the thermostabilized kan gene, in which the initial Km.sup.r supported growth only to 55.degree. C. in Thermus and not to 63.degree. C. as reported by Liao, et. al. ((1986) Isolation of a thermostable enzyme variant by cloning and selection in a thermophile. Proc. Natl. Acad. Sci. USA. 83:576-580). The regulated expression system provided herein allows for fine-tuning of thermostabilization selection experiments so that the temperature range can be regulated and controlled and cutoff temperatures for selection adjusted in subsequent rounds of mutagenesis. Some important elements of Thermus' genetic background have been previously described. The generation of mutations (Koyama, et al. (1990) Cloning and sequence analysis of tryptophan synthetase genes of an extreme thermophile, Thermus thermophilus HB27: Plasmid transfer from replica-plated Escherichia coli recombinant colonies to competent T. thermophilus cells. J. Bacteriol. 172:3490-3495; Koyama, et al. (1990) A plasmid vector for an extreme thermophile, Thermus thermophilus. FEMS Microbiol. Lett. 72:97-102; Lasa, et al. (1992) Insertional mutagenesis in the extreme thermophilic eubacteria Thermus thermophilus HB8. Molec. Microbiol. 6:1555-1564), chromosomal integration (Koyama, et al. (1990) Cloning and sequence analysis of tryptophan synthetase genes of an extreme thermophile, Thermus thermophilus HB27: Plasmid transfer from replica-plated Escherichia coli recombinant colonies to competent T. thermophilus cells. J. Bacteriol. 172:3490-3495; Koyama, et al. (1990) A plasmid vector for an extreme thermophile, Thermus thermophilus. FEMS Microbiol. Lett. 72:97-102; Lasa, et al. (1992) Insertional mutagenesis in the extreme thermophilic eubacteria Thermus thermophilus HB8. Molec. Microbiol. 6:1555-1564), plasmids (Mather, et al. (1990) Plasmid-associated aggregation in Thermus thermophilus HB8. Plasmid. 24:45-56; Hishinuma, et al. (1978) Isolation of extrachromosomasl deoxyribonucleic Acids from extremely thermophilic bacteria. Jour. of General Microbiology. 104:193-199.), and phages (Sakaki, et al. (1975) Isolation and Characterization of a Bacteriophage Infectious to an Extreme Thermophile, Thermus thermophilus HB8. J.Virol. 15:1449-1453) have also been studied. Several successful attempts to develop cloning systems using plasmids and chromosomal integration systems were demonstrated (Koyama, et al. (1986) Genetic transformation of the extreme thermophile thermos thermophilus and of other thermus spp. J. Bacteriol. 166:338-340; Lasa, et al. (1992) Development of Thermus-Escherichia Shuttle Vectors and Their Use for Expression of the Clostridium thermocellum celA Gene in Thermus thermophilus. J. Bacteriol. 174:6424-6431; Mather, et al. (1992) Development of Plasmid Cloning Vectors for Thermus thermophilus HB8: Expression of a Heterologous, Plasmid-Borne Kanamycin Nucleotidyltransferase Gene. Appl. Environ. Microbiol 58:421-425.). However, none of these provide the versatility as those provided herein.

4. Document ID: US 6207377 B1

L6: Entry 4 of 7 File: USPT

Mar 27, 2001

US-PAT-NO: 6207377
DOCUMENT-IDENTIFIER: US 6207377 B1

TITLE: Method for construction of thermus-E. coli shuttle vectors and identification of two Thermus plasmid replication origins

DATE-ISSUED: March 27, 2001

US-CL-CURRENT: 435/6; 435/252.3, 435/320.1, 435/471, 435/91.1, 536/23.1, 536/24.1

APPL-NO: 9/ 134246
DATE FILED: August 14, 1998
IN: Wayne; Jay, Xu; Shuang-yong

AB: The present invention relates to cloned DNA containing origin of DNA replication and to cloned DNA encoding repliation protein, RepT.

L6: Entry 4 of 7 File: USPT

Mar 27, 2001

DOCUMENT-IDENTIFIER: US 6207377 B1
TITLE: Method for construction of thermus-E. coli shuttle vectors and identification of two Thermus plasmid replication origins

Brief Summary Paragraph Right (1):
The present invention relates to recombinant DNA molecules encoding plasmid DNA replication origins in Thermus, as well as to shuttle vectors which contain the same.

Brief Summary Paragraph Right (4):
A Thermus-E. coli shuttle vector would be desirable if one needs to have the convenience of cloning in E. coli, isolation of DNA from E. coli for further manipulations and subsequently gene selection and expression in Thermus. Such Thermus-E. coli shuttle vectors could be used to screen, select and express thermostable proteins in Thermus. Using these vectors, a gene could, for example, be mutated within a mesophile, transferred to a thermophile, and then its encoded protein selected for increased thermostability. In this way, mesophile-thermophile shuttle-vectors can be used to conduct directed evolution, or protein engineering, on desirable gene products.

Brief Summary Paragraph Right (6):
The present invention relates to recombinant DNA molecules encoding plasmid DNA replication origins in Thermus, as well as to shuttle vectors which contain the same.

Detailed Description Paragraph Right (17):
The repeats and inverted repeats are important for pTsp45L origin of replication, because deletion of these repeats in a HindIII fragment abolished DNA replication in Thermus. The DNA sequence of pTsp45L is shown in FIG. 7. The Thermus-E. coli shuttle vector containing pTsp45L DNA replication origin was named as pUC-EKR-Tsp45L9Kb.

Detailed Description Paragraph Type 1 (8):
8. To reduce the size of the Thermus replication origin, the 4.2 kb XbaI fragment was further digested with restriction enzymes

and subcloned into pUC-EKF or pUC-EKR. One recombinant plasmid contained a 2.3 kb NheI fragment that replicates in Thermus and E. coli. This plasmid pUC-EKF-Tsp3 is a Thermus-E. coli shuttle vector.

Detailed Description Paragraph Type 1 (9):
9. One open reading frame of 1026 bp encoding a 341-amino acid protein was found within the Thermus origin. Deletion of 234 bp (78 amino acid residues) within this gene abolished the Thermus replication function. Insertion of stop codons within this gene causes premature termination and negates the Thermus transformation. Therefore it was determined that this gene (repT) is required for plasmid replication in Thermus HB27 (Pro.sup.-) cells.

Detailed Description Paragraph Type 1 (10):
10. Two Thermus promoters were found upstream of the repT gene that are important for repT expression.

5. Document ID: US 5872238 A

L6: Entry 5 of 7 File: USPT

Feb 16, 1999

US-PAT-NO: 5872238
DOCUMENT-IDENTIFIER: US 5872238 A

TITLE: Thermophile gene transfer

DATE-ISSUED: February 16, 1999

US-CL-CURRENT: 536/23.7

APPL-NO: 8/ 912794
DATE FILED: August 18, 1997

PARENT-CASE:

This application is a continuation of application Ser. No. 08/496,932, filed on Jun. 30, 1995, now abandoned which is a continuation-in-part of U.S. patent application Ser. No. 08/265,522 filed Jun. 24, 1994, now abandoned.

IN: Weber; J. Mark, Demirjian; David C., Casadaban; Malcolm J., Vonstein; Veronika, Pagratis; Nikos C.

AB: We have developed a new gene transfer system for extreme thermophiles of the genus Thermus, including Thermus flavus., using a chromosomal gene, and a thermostable derivative of the kanamycin-resistance gene (kan.sup.tr2). A plasmid mediated gene-replacement process is used to insert it into the chromosome resulting in the production of Leu.sup.- Km.sup.r transformants. This system not only allows stable, single-copy gene insertion into the chromosome of an extreme thermophile, but can be used in the thermo-genetic process described here to generate thermo-stabilized enzymes and proteins for industrial processes. This host-vector environment makes it possible to generate further thermo-stabilizing mutations in the kan gene beyond those levels previously reported.

L6: Entry 5 of 7 File: USPT

Feb 16, 1999

DOCUMENT-IDENTIFIER: US 5872238 A

TITLE: Thermophile gene transfer

Brief Summary Paragraph Right (9):
Koyama et al. (1990) A plasmid vector for an extreme thermophile, Thermus thermophilus, FEMS Microbiology Letters 72:97-102, teach a Thermus-E. coli shuttle vector carrying a tryptophan synthetase gene (trpB). This cryptic plasmid pTT8, was able to transform Thermus thermophilus. The authors point out that a plasmid vector carrying trpBA was not suitable for selection since the cloned DNA fragment recombined with the chromosomal counterpart at high frequency.

Brief Summary Paragraph Right (17):
Lasa et al. (1992a) Development of Thermus-Escherichia Shuttle Vectors and Their Use for Expression of the Clostridium thermocellum celA Gene in Thermus thermophilus, J. of Bacteriology 174:6424-6431, teach the self-selection of undescribed origins of replication from cryptic plasmids from uncharacterized Thermus spp. and Thermus aquaticus are isolated and cloned into E. coli vectors. Plasmids were constructed with these origins, pLU1 to pLU4 from T. aquaticus, and pMY1 to pMY3 from Thermus spp. The plasmids then had a modified form of the cellulase gene (celA) from Clostridium thermocellum and were expressed in E. coli with the signal peptide from the S-layer gene from T. thermophilus. Transformation back into T. thermophilus allowed for expression at 70.degree. C.

Other Reference Publication (11):
Lasa et al. (1992a) Development of Thermus-Escherichia Shuttle Vectors and Their Use for Expression of the Clostridium thermocellum celA Gene in Thermus thermophilus, J. of Bacteriology 174:6424-6431.

6. Document ID: US 5786174 A

L6: Entry 6 of 7 File: USPT

Jul 28, 1998

US-PAT-NO: 5786174
DOCUMENT-IDENTIFIER: US 5786174 A

TITLE: Thermophile gene transfer

DATE-ISSUED: July 28, 1998

US-CL-CURRENT: 435/69.1; 435/463, 530/350, 536/23.1

APPL-NO: 8/ 790309
DATE FILED: January 28, 1997

PARENT-CASE:

This application is a continuation of application Ser. No. 08/265,522, filed on 24 Jun., 1994, now abandoned.

IN: Weber; J. Mark, Demirjian; David C., Casadaban; Malcolm J., Pagratis; Nikos C., Vonstein; Veronika

AB: We have developed a new gene transfer system for extreme thermophiles of the genus Thermus, including Thermus flavus., using a chromosomal gene, and a thermostable derivative of the kanamycin-resistance gene (kan.sup.tr2). A plasmid mediated gene-replacement process is used to insert it into the chromosome resulting in the production of Leu.sup.- Km.sup.r transformants. This system not only allows stable, single-copy gene insertion into the chromosome of an extreme thermophile, but can be used in the

thermo-genetic process described here to generate
thermo-stabilized enzymes and proteins for industrial processes. This host-vector environment makes it possible to
generate further thermo-stabilizing mutations in the kan gene beyond those levels previously reported.

L6: Entry 6 of 7 File: USPT Jul 28, 1998

DOCUMENT-IDENTIFIER: US 5786174 A
TITLE: Thermophile gene transfer

Brief Summary Paragraph Right (10):
Koyama et al. (1990) A plasmid vector for an extreme thermophile, Thermus thermophilus, FEMS Microbiology Letters 72:97-102, teach a Thermus-E. coli shuttle vector carrying a tryptophan synthetase gene (trpB). This cryptic plasmid pTT8, was able to transform Thermus thermophilus. The authors point out that a plasmid vector carrying trpBA was not suitable for selection since the cloned DNA fragment recombined with the chromosomal counterpart at high frequency.

Brief Summary Paragraph Right (18):
Lasa et al. (1992a) Development of Thermus-Escherichia Shuttle Vectors and Their Use for Expression of the Clostridium thermocellum celA Gene in Thermus thermophilus, J. of Bacteriology 174:6424-6431, teach the self-selection of undescribed origins of replication from cryptic plasmids from uncharacterized Thermus spp. and Thermus aquaticus are isolated and cloned into E. coli vectors. Plasmids were constructed with these origins, pLU1 to pLU4 from T. aquaticus, and pMY1 to pMY3 from Thermus spp. The plasmids then had a modified form of the cellulase gene (celA) from Clostridium thermocellum and were expressed in E. coli with the signal peptide from the S-layer gene from T. thermophilus. Transformation back into T. thermophilus allowed for expression at 70.degree. C.

Other Reference Publication (18):
Lasa et al. (1992a) Development of Thermus-Escherichia Shuttle Vectors and Their Use for Expression of the Clostridium thermocellum celA Gene in Thermus thermophilus, J. of Bacteriology 174:6424-6431.

7. Document ID: US 5120658 A

L6: Entry 7 of 7 File: USPT Jun 9, 1992

US-PAT-NO: 5120658
DOCUMENT-IDENTIFIER: US 5120658 A

TITLE: Thermostable tryptophan synthetase gene and extremely thermophilic plasmid vector incorporating said gene

DATE-ISSUED: June 9, 1992

US-CL-CURRENT: 435/320.1; 435/108, 435/183, 435/252.3, 435/69.1, 435/71.2, 435/91.41, 536/23.2

APPL-NO: 7/ 329765
DATE FILED: March 28, 1989

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	63-163779	June 30, 1988

IN: Koyama; Yoshinori, Furukawa; Kensuke, Tomizuka; Noboru

AB: A DNA segment, specifically a thermostable tryptophan synthetase gene originating in the strain of extremely thermophilic Thermus aquaticus T2, characterized by the restriction enzyme map of FIG. 1, and not cleaved by specific restriction enzymes., An extremely thermophilic plasmid vector pYK 105, having the DNA segment and an Escherichia coli plasmid vector pUC 13 incorporated in a cryptic plasmid pTT8.

L6: Entry 7 of 7 File: USPT Jun 9, 1992

DOCUMENT-IDENTIFIER: US 5120658 A
TITLE: Thermostable tryptophan synthetase gene and extremely thermophilic plasmid vector incorporating said gene

Detailed Description Paragraph Right (23):
The plasmid pYK 105 separated from the transformed strain possesses the structure illustrated at the bottom of FIG. 4. Since it possesses the pUC 13 plasmid, it constitutes a shuttle vector which can replicate not only in the microorganism of genus Thermus but also in the Escherichia coli. The selection of the transformed strain is attained by virtue of the tolerance to Ampicillin in the case of the Escherichia coli and the complementation of the tryptophan-demanding property in the case of the thermophilus strain of Thermus thermophilus HB 27 trp.sup.-. The pYK 105 is the first selectable plasmid vector produced with a microorganism of genus Thermus.

Detailed Description Paragraph Right (59):
When this culture was continued at 70.degree. C. for two days, there was obtained a transformed strain of Thermus thermophilus HB 27 trp.sup.- (pYK 105) no longer demanding tryptophan. The plasmid pYK 105 separated from the transformed strain possessed the structure illustrated at the bottom of FIG. 4. Since it possessed a pUC 13 plasmid, it constituted a shuttle vector which can replicate not only in the microorganism of genus Thermus but also in the Escherichia coli. The selection of the transformed strain could be effected by virtue of the ampicillin resistance in the case of the Escherichia coli and by virtue of the complementation of the tryptophan-demanding property in the case of the thermophilic strain of Thermus thermophilus HB 27 trp.sup.-. The pYK 105 is the first selectable plasmid vector constructed for the microorganism of genus Thermus.

09/664186
AMPB

=> s thermus
L1 8444 THERMUS

=> s shuttle
L2 33438 SHUTTLE

=> s rept
L3 1317 REPT

=> s promoter
L4 385651 PROMOTER

=> s l1 and l2

L5 46 L1 AND L2

=> dup rem l5
PROCESSING COMPLETED FOR L5
L6 18 DUP REM L5 (28 DUPLICATES REMOVED)

=> d l6 ibib abs 1-18

L6 ANSWER 1 OF 18 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 2002-269188 [31] WPIDS
DOC. NO. CPI: C2002-079914
TITLE: Producing transformed microorganism, preferably Bacillus, involves selecting competent microorganism, producing DNA construct in vitro, and directly transforming the microorganism with the DNA construct.
DERWENT CLASS: B04 D16
INVENTOR(S): DIAZ-TORRES, M R; LEE, E W; MORRISON, T B; SCHELLENBERGER, V; SELIFONOVA, O V
PATENT ASSIGNEE(S): (GEMV) GENENCOR INT INC
COUNTRY COUNT: 95
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2002014490	A2	20020221	(200231)*	EN	48

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2002014490	A2	WO 2001-US25166	20010810

PRIORITY APPLN. INFO: US 2000-224948P 20000811
AN 2002-269188 [31] WPIDS
AB WO 200214490 A UPAB: 20020516
NOVELTY - Producing (M1) a transformed microorganism, preferably Bacillus,
involves selecting a competent microorganism, producing a DNA construct in
vitro, and directly transforming the microorganism with the DNA construct
such that the DNA construct becomes integrated into a chromosome of the microorganism.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the
following:
(1) a library of mutants (I) produced by M1;
(2) directed evolution (M2) of a sequence in the host cell chromosome, involves in vitro mutagenesis of a DNA construct, direct transformation of the mutagenized sequence into a competent host cell, screening for, or selection of, mutants possessing or exhibiting a desired property, and repeating the above mentioned steps for one or more rounds; and
(3) constructing (M3) a sequence of interest at a target sequence of a selected microorganism, where the target sequence includes a residing marker, involves assembling a DNA construct in vitro comprising an incoming sequence, a selectable marker, and two flanking sequences which
are homologous to sequences of the target sequence, where the selectable marker of the DNA construct is different than the residing marker of the microorganism, transforming the microorganism with the DNA construct under
conditions permitting the incoming sequence and selectable marker to inactivate the residing marker, and selecting for transformants that include the selectable marker, and repeating the above mentioned steps, where with each repetition of the DNA construct comprises a selectable marker different from the selectable marker in the previous step and the selectable marker of the previous step acts as the residing marker in the microorganisms.
USE - M1 is useful for producing a transformed microorganisms selected from Acinetobacter, ***Thermus*** , Deinococcus, Radiodurans,
and Bacillus, preferably Bacillus, where the Bacillus is a super-competent strain, preferably a Pxyl-comK strain (claimed).
ADVANTAGE - M1 transforms the DNA constructs into the microorganism
with good efficiency, and allows for the generation of large libraries. M1 provides in vitro construction and direct transformation into Bacillus, without the use of any intervening microorganisms. M1 does not require antibiotic or other selectable marker to maintain the plasmid in the cells, which is undesirable for production strains and constrains choice of screening conditions. M1 allows evolution of single copy genes of a strain, and prevents variations in copy number which skews a library.
Dwg.0/15

L6 ANSWER 2 OF 18 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2002255802 IN-PROCESS
DOCUMENT NUMBER: 21911539 PubMed ID: 11914489
TITLE: Comparative analysis of space-grown and earth-grown crystals of an aminoacyl-tRNA synthetase: space-grown crystals are more useful for structural determination.
AUTHOR: Ng Joseph D; Sauter Claude; Lorber Bernard; Kirkland Natalie; Arnez John; Giege Richard
CORPORATE SOURCE: Departement Mecanismes et Macromolecules de la Synthese
Proteique et Cristallogenese, UPR 9002, Institut de Biologie Moleculaire et Cellulaire du CNRS, 15 Rue Rene Descartes, F-67084 Strasbourg CEDEX, France.
SOURCE: ACTA CRYSTALLOGRAPHICA. SECTION D: BIOLOGICAL
CRYSTALLOGRAPHY, (2002 Apr) 58 (Pt 4) 645-52.
Journal code: 9305878. ISSN: 0907-4449.
PUB. COUNTRY: Denmark
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: IN-PROCESS; NONINDEXED; Priority Journals
OTHER SOURCE: PDB-110W; PDB-R110WSF
ENTRY DATE: Entered STN: 20020509
Last Updated on STN: 20020509
AB Protein crystallization under microgravity aims at benefiting from the quasi-absence of convection and sedimentation to favor well ordered crystal nucleation and growth. The dimeric multidomain enzyme aspartyl-tRNA synthetase from ***Thermus*** thermophilus has been crystallized within dialysis reactors of the Advanced Protein Crystallization Facility in the laboratory on earth and under microgravity aboard the US Space ***Shuttle*** . A strictly comparative crystallographic analysis reveals that the crystals grown in space are superior in every respect to control crystals prepared in otherwise identical conditions on earth. They diffract X-rays more intensely and have a lower mosaicity, facilitating the process of protein structure determination. Indeed, the electron-density map calculated from diffraction data of space-grown crystals contains considerably more detail. The resulting three-dimensional structure model at 2.0 A resolution is more accurate than that produced in parallel using the data originating from earth-grown crystals. The major differences between the structures, including the better defined amino-acid side chains and the higher order of bound water molecules, are emphasized.

L6 ANSWER 3 OF 18 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:221921 HCAPLUS

DOCUMENT NUMBER: 134:247953
TITLE: Replication origins and proteins of plasmids of the thermophilic bacterium ***Thermus*** and the construction of ***Thermus*** -E. coli ***shuttle*** vectors
INVENTOR(S): Wayne, Jay; Xu, Shuang-Yong
PATENT ASSIGNEE(S): New England Biolabs, Inc., USA
SOURCE: U.S., 32 pp.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6207377	B1	20010327	US 1998-134246	19980814

AB Replication origins and functions of two plasmids of the thermophilic bacterium ***Thermus*** YS45 are described. Two genes, oriT of pTsp45S and parA of pTsp45L, that are essential for replication are cloned and characterized. These functions may be useful in the construction of ***Thermus*** -Escherichia coli ***shuttle*** vectors.
REFERENCE COUNT: 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 4 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
ACCESSION NUMBER: 2001:462444 BIOSIS
DOCUMENT NUMBER: PREV200100462444
TITLE: Production of recombinant alpha-galactosidases in ***Thermus*** thermophilus.
AUTHOR(S): Fridjonsson, Olafur (1); Mattes, Ralf
CORPORATE SOURCE: (1) Prokaria Ltd., Gylfaflot 5, 112, Reykjavik: olafur@prokaria.com Iceland
SOURCE: Applied and Environmental Microbiology, (September, 2001)
Vol. 67, No. 9, pp. 4192-4198. print.
ISSN: 0099-2240.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A ***Thermus*** thermophilus selector strain for production of thermostable and thermoactive alpha-galactosidase was constructed. For this purpose, the native alpha-galactosidase gene (agaT) of T. thermophilus TH125 was inactivated to prevent background activity. In our first attempt, insertional mutagenesis of agaT by using a cassette carrying a kanamycin resistance gene led to bacterial inability to utilize melibiose (alpha-galactoside) and galactose as sole carbohydrate sources due to a polar effect of the insertional inactivation. A Gal+ phenotype was assumed to be essential for growth on melibiose. In a Gal- background, accumulation of galactose or its metabolite derivatives produced from melibiose hydrolysis could interfere with the growth of the host strain harboring recombinant alpha-galactosidase. Moreover, the AgaT- strain had to be Kms for establishment of the plasmids containing alpha-galactosidase genes and the kanamycin resistance marker. Therefore, a suitable selector strain (AgaT- Gal+ Kms) was generated by applying integration mutagenesis in combination with phenotypic selection. To produce heterologous alpha-galactosidase in T. thermophilus, the isogenes agaA and agaB of Bacillus stearothermophilus KVE36 were cloned into an Escherichia coli- ***Thermus*** ***shuttle*** vector. The region containing the E. coli plasmid sequence (pUC-derived vector) was deleted before transformation of T. thermophilus with the recombinant plasmids. As a result, transformation efficiency and plasmid stability were improved. However, growth on minimal agar medium containing melibiose was achieved only following random selection of the clones carrying a plasmid-based mutation that had promoted a higher copy number and greater stability of the plasmid.

L6 ANSWER 5 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 3
ACCESSION NUMBER: 2001:186597 BIOSIS
DOCUMENT NUMBER: PREV200100186597
TITLE: Characterization of the minimal replicon of a cryptic Deinococcus radiodurans SARK plasmid and development of versatile Escherichia coli-D. radiodurans ***shuttle*** vectors.
AUTHOR(S): Meima, Rob; Lidstrom, Mary E. (1)
CORPORATE SOURCE: (1) Department of Chemical Engineering, University of Washington, Seattle, WA, 98195-1750: lidstrom@u.washington.edu USA
SOURCE: Applied and Environmental Microbiology, (September, 2000)
Vol. 66, No. 9, pp. 3856-3867. print.
ISSN: 0099-2240.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The nucleotide sequence of a 12-kb fragment of the cryptic Deinococcus radiodurans SARK plasmid pUE10 was determined, in order to direct the development of small, versatile cloning systems for Deinococcus. Annotation of the sequence revealed 12 possible open reading frames. Among these are the repU and resU genes, the predicted products of which share similarity with replication proteins and site-specific resolvases, respectively. The products of both genes were demonstrated using an overexpression system in Escherichia coli. RepU was found to be required for replication, and ResU was found to be required for stable maintenance of pUE10 derivatives. Gel shift analysis using purified His-tagged RepU identified putative binding sites and suggested that RepU may be involved in both replication initiation and autoregulation of repU expression. In addition, a gene encoding a possible antirestriction protein was found, which was shown to be required for high transformation frequencies. The arrangement of the replication region and putative replication genes for this plasmid from D. radiodurans strain SARK is similar to that for plasmids found in ***Thermus*** but not to that for the 45.7-kb plasmid found in D. radiodurans strain R1. The minimal region required for autonomous replication in D. radiodurans was determined by sequential deletion of segments from the 12-kb fragment. The resulting minimal replicon, which consists of approximately 2.6 kb, was used for the construction of a ***shuttle*** vector for E. coli and D. radiodurans. This vector, pRAD1, is a convenient general-purpose cloning vector. In addition, pRAD1 was used to generate a promoter probe vector, and a plasmid containing lacZ and a Deinococcus promoter was shown to efficiently express LacZ.

L6 ANSWER 6 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4
ACCESSION NUMBER: 1999:199329 BIOSIS
DOCUMENT NUMBER: PREV199900199329
TITLE: Dissimilatory reduction of Fe(III) and other electron acceptors by a ***Thermus*** isolate.
AUTHOR(S): Kieft, T. L. (1); Fredrickson, J. K.; Onstott, T. C.; Gorby, Y. A.; Kostandarithes, H. M.; Bailey, T. J.; Kennedy, D. W.; Li, S. W.; Plymale, A. E.; Spadoni, C. M.; Gray, M. S.
CORPORATE SOURCE: (1) Department of Biology, New Mexico Institute of Mining and Technology, Socorro, NM, 87801 USA
SOURCE: Applied and Environmental Microbiology, (March, 1999) Vol. 65, No. 3, pp. 1214-1221.
ISSN: 0099-2240.
DOCUMENT TYPE: Article
LANGUAGE: English
AB A thermophilic bacterium that can use O2, NO3-, Fe(III), and S0 as terminal electron acceptors for growth was isolated from groundwater sampled at a 3.2-km depth in a South African gold mine. This organism, designated SA-01, clustered most closely with members of the genus ***Thermus*** , as determined by 16S rRNA gene (rDNA) sequence analysis. The 16S rDNA sequence of SA-01 was >98% similar to that of ***Thermus***

strain NMX2 A.1, which was previously isolated by other investigators from
a thermal spring in New Mexico. Strain NMX2 A.1 was also able to reduce
Fe(HI) and other electron acceptors. Neither SA-01 nor NMX2 A.1 grew fermentatively, i.e., addition of an external electron acceptor was required for anaerobic growth. ***Thermus*** strain SA-01 reduced soluble Fe(III) complexed with citrate or nitrilotriacetic acid (NTA); however, it could reduce only relatively small quantities (0.5 mM) of hydrous ferric oxide except when the humic acid analog 2,6-anthraquinone disulfonate was added as an electron ***shuttle*** , in which case 10 mM Fe(III) was reduced. Fe(III)-NTA was reduced quantitatively to Fe(II);
reduction of Fe(III)-NTA was coupled to the oxidation of lactate and supported growth through three consecutive transfers. Suspensions of ***Thermus*** strain SA-01 cells also reduced Mn(IV), Co(III)-EDTA,
Cr(VI), and U(VI). Mn(IV)-oxide was reduced in the presence of either lactate or H2. Both strains were also able to mineralize NTA to CO2 and to
couple its oxidation to Fe(III) reduction and growth. The optimum temperature for growth and Fe(III) reduction by ***Thermus*** strains SA-01 and NMX2 A.1 is approximately 65degreeC; their optimum pH is 6.5 to
7.0. This is the first report of a ***Thermus*** sp. being able to couple the oxidation of organic compounds to the reduction of Fe, Mn, or S.

L6 ANSWER 7 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
5
ACCESSION NUMBER: 2000:51935 BIOSIS
DOCUMENT NUMBER: PREV200000051935
TITLE: Ribosomal gene disruption in the extreme thermophile ***Thermus*** thermophilus HB8. Generation of a mutant lacking ribosomal protein S17.
AUTHOR(S): Simitsopoulou, Maria; Avila, Horacio; Franceschi, Francois
(1)
CORPORATE SOURCE: (1) Max-Planck-Institut fuer Molekulare Genetik, AG
Ribosomen, Ihnestrasse 73, Berlin, 14195 Germany
SOURCE: European Journal of Biochemistry, (Dec., 1999) Vol. 266, No. 2, pp. 524-532.
ISSN: 0014-2956.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB S17 is a primary rRNA-binding protein which has been implicated in ribosome assembly and translational fidelity. We describe the generation and biochemical characterization of an S17 minus ribosomal mutant, a ribosomal protein-lacking mutant obtained in ***Thermus*** thermophilus HB8. The S17 mutant was obtained by insertional inactivation
of the target gene with the kanamycin adenyl transferase (kat) gene, making use of a ***Thermus*** -Escherichia ***shuttle*** vector and
the natural ability of ***Thermus*** to transform. In the final construct used to transform ***Thermus*** cells, the S17 coding region was replaced with the kat gene cloned in-frame with the first three amino acids of S17. Hence, in vivo transcription of the kat gene was under the control of the ribosomal operon promoter. As in Escherichia coli, the ***Thermus*** S17 mutant exhibited a temperature-sensitive phenotype.
Two-dimensional PAGE, Western blot, and ELISA confirmed the absence of S17
from the mutant ribosomes. Sucrose-gradient profiles of mutant cells showed a clear separation and normal proportions of 50S and 30S subunits and a normal ratio between them. In addition, the S17 mutant showed the presence of a 20S peak representing assembly-defective particles. The successful re-incorporation of protein S17 into the mutant ribosomes was demonstrated when reconstitution with isolated S17 was performed at 60 degreeC.

L6 ANSWER 8 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
6
ACCESSION NUMBER: 2000:27289 BIOSIS
DOCUMENT NUMBER: PREV200000027289
TITLE: A high-transformation-efficiency cloning vector for ***Thermus*** thermophilus.
AUTHOR(S): de Grado, Myriam; Castan, Pablo; Berenguer, Jose (1)
CORPORATE SOURCE: (1) Centro de Biologa Molecular "Severo Ochoa", UAM-CSIC,
Universidad Autonoma de Madrid, 28049, Madrid Spain
SOURCE: Plasmid, (Nov., 1999) Vol. 42, No. 3, pp. 241-245.
ISSN: 0147-619X.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The cloning vector pMK18 was developed through the fusion of the minimal
replicative region from an indigenous plasmid of ***Thermus*** sp. ATCC27737, a gene cassette encoding a thermostable resistance to kanamycin, and the replicative origin and multiple cloning site of pUC18. Plasmid pMK18 showed transformation efficiencies from 108 to 109 per microgram of plasmid in ***Thermus*** thermophilus HB8 and HB27, both
by natural competence and by electroporation. We also show that T. thermophilus HB27 can take pMK18 modified by the Escherichia coli methylation system with the same efficiency as its own DNA. To demonstrate
its usefulness as a cloning vector, a gene encoding the beta-subunit of a thermostable nitrate reductase was directly cloned in T. thermophilus HB27
from a gene library. Its further transfer to E. coli also proved its utility as a ***shuttle*** vector.

L6 ANSWER 9 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
7
ACCESSION NUMBER: 1997:414348 BIOSIS
DOCUMENT NUMBER: PREV199799706391
TITLE: A new ***Thermus*** -Escherichia coli ***shuttle*** integration vector system.
AUTHOR(S): Tamakoshi, Masatada; Uchida, Manabu; Tanabe, Kazuhiro;
Fukuyama, Shiro; Yamagishi, Akihiko (1); Oshima, Tairo
CORPORATE SOURCE: (1) Dep. Mol. Biol., Tokyo Univ. Pharmacy Life Sci., 1432
Horinouchi, Hachioji, Tokyo 192-03 Japan
SOURCE: Journal of Bacteriology, (1997) Vol. 179, No. 15, pp. 4811-4814.
ISSN: 0021-9193.
DOCUMENT TYPE: Article
LANGUAGE: English
AB We established a ***Thermus*** thermophilus strain in which the pyrE
gene (coding for orotate phosphoribosyltransferase of the pyrimidine biosynthetic pathway) was totally deleted. We also constructed an integration vector, which consisted of the Escherichia coli plasmid vector pBluescript and a 2.1-kb segment of the T. thermophilus leu operon sequence, for the integration of a foreign gene into a chromosome of the thermophile. pyrE and leuB genes were used as probes to test the integration vector. The integration vector pINV, bearing the pyrE gene, transformed the DELTA-pyrE strain at a frequency of 6 times 10-5 through a
single crossover event. The leuB gene could also be used as another marker
of the integration vector system. The vector could be integrated at the expected site. By digesting the chromosomal DNA of the T. thermophilus transformants with a unique restriction enzyme, the vector could be recovered into E. coli after the recircularization in vitro. The kanamycin nucleotidyltransferase gene could be successfully expressed in the thermophile by using pINV.

L6 ANSWER 10 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
8
ACCESSION NUMBER: 1997:506817 BIOSIS
DOCUMENT NUMBER: PREV199799806020
TITLE: Lumenal proteins involved in respiratory electron transport in the cyanobacterium Synechocystis sp. PCC6803.
AUTHOR(S): Manna, Pradip; Vermaas, Wim (1)
CORPORATE SOURCE: (1) Molecular Cellular Biol. Program, Dep. Botany, Cent.

Study Early Events Photosynthesis, Arizona State Univ., Box 871601, Tempe, AZ 85287-1601 USA
SOURCE: Plant Molecular Biology, (1997) Vol. 35, No. 4, pp. 407-416.
ISSN: 0167-4412.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Cyanobacterial thylakoids catalyze both photosynthetic and respiratory activities. In a photosystem I-less Synechocystis sp. PCC 6803 strain, electrons generated by photosystem II appear to be utilized by cytochrome oxidase. To identify the lumenal electron carriers (plastocyanin and/or cytochromes c-553, c-550, and possibly c-M) that are involved in transfer of photosystem II-generated electrons to the terminal oxidase, deletion constructs for genes coding for these components were introduced into a photosystem I-less Synechocystis sp. PCC 6803 strain, and electron flow out of photosystem II was monitored in resulting strains through chlorophyll fluorescence yields. Loss of cytochrome c-553 or plastocyanin, but not of cytochrome c-550, decreased the rate of electron flow out of photosystem II. Surprisingly, cytochrome c-M could not be deleted in a photosystem I-less background strain, and also a double-deletion mutant lacking both plastocyanin and cytochrome c-553 could not be obtained. Cytochrome c-M has some homology with the cytochrome c-binding regions of the cytochrome caa-3-type cytochrome oxidase from Bacillus spp. and ***Thermus*** thermophilus. We suggest that cytochrome c-M is a component of cytochrome oxidase in cyanobacteria that serves as redox intermediate between soluble electron carriers and the cytochrome aa-3 complex, and that either plastocyanin or cytochrome c-553 can ***shuttle*** electrons from the cytochrome b-6f complex to cytochrome c-M.

L6 ANSWER 11 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 9
ACCESSION NUMBER: 1997:453208 BIOSIS
DOCUMENT NUMBER: PREV199799752411
TITLE: Identification of a thermophilic plasmid origin and its cloning within a new ***Thermus*** -E. coli ***shuttle*** vector.
AUTHOR(S): Wayne, Jay; Xu, Shuang-Yong (1)
CORPORATE SOURCE: (1) New Engl. Biolabs, 32 Tozer Road, Beverly, MA 01915 USA
SOURCE: Gene (Amsterdam), (1997) Vol. 195, No. 2, pp. 321-328.
ISSN: 0378-1119.
DOCUMENT TYPE: Article
LANGUAGE: English
AB A pUC19-based vector has been generated for selecting functional thermophilic origins (oris) of ***Thermus*** ssp. Once combined with thermophilic DNA, the vector can be amplified in ampicillin resistant (Ap-R) E. coli, prior to transformation and kanamycin (Km) selection in ***Thermus*** thermophilus. The Km-R ***Thermus*** transformants replicate any newly-formed ***shuttle*** vectors via introduced thermophilic oris. Using this "ori-selecting" vector, three novel thermophilic oris were cloned from randomly digested ***Thermus*** cryptic plasmid DNA. These ***shuttle*** vectors are useful for genetic analyses, as well as protein engineering within thermophiles. The smallest ori-containing sequence of 4.2 kb has been subcloned, sequenced, and further refined to 2.3 kb. A significant ORF of 341 amino acids (aa), with a ***Thermus*** promoter and RBS, is found within the thermophilic ori. Deleting part of this ORF abolishes the ***shuttle*** vector's ability to replicate in T. thermophilus. Therefore, we postulate that this ORF encodes a replication protein (Rep) necessary for thermophilic plasmid replication. The thermophilic ori also contains two sequences which resemble DnaA boxes.

L6 ANSWER 12 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 10
ACCESSION NUMBER: 1993:4035 BIOSIS
DOCUMENT NUMBER: PREV199395004035
TITLE: Development of ***Thermus*** and Escherichia ***shuttle*** vectors and their use for expression of the Clostridium thermocellum celA gene in ***Thermus*** thermophilus.
AUTHOR(S): Lasa, Inigo; De Grado, M.; De Pedro, M. A.; Berenguer, Jose (1)
CORPORATE SOURCE: (1) Centro de Biologie Molecular, Universidad Autonoma de Madrid-Consejo Superior de Investigaciones Cientificas, 28049 Madrid Spain
SOURCE: Journal of Bacteriology, (1992) Vol. 174, No. 20, pp. 6424-6431.
ISSN: 0021-9193.
DOCUMENT TYPE: Article
LANGUAGE: English
AB We describe the self-selection of replication origins of undescribed cryptic plasmids from ***Thermus*** aquaticus Y-VII-51B (ATCC 25105) and a ***Thermus*** sp. strain (ATCC 27737) by random insertion of a thermostable kanamycin adenyltransferase cartridge. Once selected, these autonomous replication origins were cloned into the Escherichia coli vector pUC9 or pUC19. The bifunctional plasmids were analyzed for their sizes, relationships, and properties as ***shuttle*** vectors for ***Thermus*** -Escherichia cloning. Seven different vectors with diverse kanamycin resistance levels, stabilities, transformation efficiencies, and copy numbers were obtained. As a general rule, those from T. aquaticus (pLU1 to pLU4) were more stable than those from the ***Thermus*** sp. (pMY1 to pMY3). To probe their usefulness, we used one of the plasmids (pMY1) to clone in E. coli a modified form of the cellulase gene (celA) from Clostridium thermocellum in which the native signal peptide was replaced in vitro by that from the S-layer gene of T. thermophilus HB8. The hybrid product was expressed and exported by E. coli. When the gene was transferred by transformation into T. thermophilus, the cellulase protein was also expressed and secreted at 70 degree C.

L6 ANSWER 13 OF 18 MEDLINE
ACCESSION NUMBER: 90363893 MEDLINE
DOCUMENT NUMBER: 90363893 PubMed ID: 2203048
TITLE: Molecular structures and evolution of mouse isozyme genes functioning in the malate-aspartate ***shuttle*** .
AUTHOR: Shimada K; Joh T; Ding S H; Choudhury B K; Setoyama C
CORPORATE SOURCE: Department of Biochemistry, Kumamoto University Medical School, Japan.
SOURCE: PROGRESS IN CLINICAL AND BIOLOGICAL RESEARCH, (1990) 344 139-58.
Journal code: PZ5; 7605701. ISSN: 0361-7742.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199010
ENTRY DATE: Entered STN: 19901109
Last Updated on STN: 19980206
Entered Medline: 19901003
AB To examine molecular mechanisms of transcription of mammalian isozyme genes functioning in the malate-aspartate ***shuttle*** and to observe structural and evolutionary relationships, we investigated gene organizations of cAspAT and mAspAT, and cMDH and mMDH, and isolated and characterized cDNAs and genomic DNAs for these isozymes in mice. The deduced amino acid sequences of mouse cAspAT and mAspAT showed about 47%, and those of mouse cMDH and mMDH, about 23% overall homology. Surprisingly, the homology between the mouse cMDH and thermophilic bacterial MDH, as well as the homology between the mouse mMDH and E. coli MDH, markedly exceeds the intraspecies sequence homology between mMDH and cMDH from mice. The first duplication of a common ancestral MDH gene should thus have occurred long before the emergence of the eukaryotic cells, and subsequently, the mammalian mMDH and E. coli MDH genes have evolved from one of the duplicates. The mammalian cMDH and ***Thermus*** flavus MDH genes have no doubt evolved from one of the other

duplicates.
Moreover, structural organizations of the two-pairs of isozyme genes indicated that introns antedate the divergence of these mitochondrial and cytosolic isozyme genes. The 5' ends of all four isozyme genes lacked the TATA and CAAT boxes characteristic of eukaryotic promoters but did contain
G + C-rich sequences and multiple transcription-initiation sites. We found several highly conserved regions in the 5' flanking sequences between mAspAT and cAspAT, between mMDH and mAspAT, and between cMDH and cAspAT
genes.

L6 ANSWER 14 OF 18 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
11
ACCESSION NUMBER: 1991:34771 BIOSIS
DOCUMENT NUMBER: BR40:11751
TITLE: A PLASMID VECTOR FOR AN EXTREME THERMOPHILE ***THERMUS***
-THERMOPHILUS.
AUTHOR(S): KOYAMA Y; ARIKAWA Y; FURUKAWA K
CORPORATE SOURCE: FERMENTATION RES. INST., AIST, MITI, TSUKUBA SCI. CITY,
IBARAKI 305, JAPAN.
SOURCE: FEMS Microbiol. Lett., (1990) 72 (1-2), 97-102.
CODEN: FMLED7. ISSN: 0378-1097.
FILE SEGMENT: BR; OLD
LANGUAGE: English

L6 ANSWER 15 OF 18 MEDLINE
ACCESSION NUMBER: 91130853 MEDLINE
DOCUMENT NUMBER: 91130853 PubMed ID: 2283046
TITLE: A plasmid vector for an extreme thermophile, ***Thermus*** thermophilus.
AUTHOR: Koyama Y; Arikawa Y; Furukawa K
CORPORATE SOURCE: Fermentation Research Institute, AIST, MITI, Tsukuba
Science City, Ibaraki, Japan.
SOURCE: FEMS MICROBIOLOGY LETTERS, (1990 Oct) 60 (1-2) 97-101.
Journal code: FML; 7705721. ISSN: 0378-1097.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199103
ENTRY DATE: Entered STN: 19910405
Last Updated on STN: 19910405
Entered Medline: 19910321
AB The host-vector system for an extreme thermophile, ***Thermus*** thermophilus HB27, was developed. The host strain has a mutation in tryptophan synthetase gene (trpB), and the mutation was determined to be a
missense mutation by DNA sequence analysis. A ***Thermus*** -E. coli
shuttle vector pYK109 was constructed. pYK109 consists of ***Thermus*** cryptic plasmid pTT8, tryptophan synthetase gene (trpB) of
Thermus T2 and E. coli plasmid vector pUC13. pYK109 transformed T.
thermophilus HB27 trpB5 to Trp+ at a frequency of 10(6) transformants per
microgram DNA.

L6 ANSWER 16 OF 18 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1990:2020 HCAPLUS
DOCUMENT NUMBER: 112:2020
TITLE: Plasmid composite for Escherichia coli and thermophilic bacteria
INVENTOR(S): Kawamata, Akiko; Fujita, Shozo; Asano, Takaharu; Hataya, Takafumi
PATENT ASSIGNEE(S): Fujitsu Ltd., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 3 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 01091784	A2	19890411	JP 1987-245852	19871001

AB ***Shuttle*** plasmids suitable for gene cloning in thermophilic bacteria at high temp. are prepd. from pBR322 of Escherichia coli and cryptic plasmid pTT8 of highly-thermophilic bacteria, e.g. ***Thermus***
thermophilus. Plasmid pBTT1 and pBTT3 were prepd. from the two plasmids
described above. The plasmids were maintained stably for >20 generations
in T. thermophilus.

L6 ANSWER 17 OF 18 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1989:34809 HCAPLUS
DOCUMENT NUMBER: 110:34809
TITLE: A chloramphenicol-selectable plasmid and its construction for cloning in thermophilic bacteria
INVENTOR(S): Yasuda, Hachiro; Fujita, Shozo; Asano, Takaharu; Kawamata, Akiko
PATENT ASSIGNEE(S): Fujitsu Ltd., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 3 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 63148986	A2	19880621	JP 1986-296813	19861215

AB The chloramphenicol acetyl transferase gene (CAT) is first successfully inserted into the plasmid pTT8 of ***Thermus*** thermophilus to obtain
a chlorampheicol-selectable plasmid useful for cloning in thermophilic bacteria. A ***shuttle*** vector (no name given) for T. thermophilus and Escherichia coli was constructed by inserting in pTT8 a BamHI fragment
contg. the ampicillin-resistance gene of pBR322 and the CAT gene of plasmid pC194.

L6 ANSWER 18 OF 18 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1983:66396 HCAPLUS
DOCUMENT NUMBER: 98:66396
TITLE: Construction of various host vector systems and the variation of enzyme levels
AUTHOR(S): Sakaguchi, K.
CORPORATE SOURCE: Lab. Microbiol. Chem., Mitsubishi-Kasei Inst. Life
Sci., Tokyo, Japan
SOURCE: Enzyme Eng. (1982), 6, 479-89
CODEN: ENENDT; ISSN: 0094-8500
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Various published and unpublished expts. in the genetic engineering of several species are discussed, esp. the use of genetic engineering in the modification of enzyme expression. Expts. discussed include: the cloning of the Bacillus subtilis gene leu region on ***shuttle*** vector plasmids and subsequent expression in a Bacillus subtilis recE4 mutant, the increased activity of tryptophan synthetase [9014-52-2] encoded by Escherichia coli DNA cloned in Pseudomonas aeruginosa, the cloning and expression of the leuB and leuC genes which encode .beta.-isopropylmalate
dehydrogenase [9030-97-1] and .alpha.-isopropylmate isomerase [50812-24-3], resp. of ***Thermus*** thermophilus in E. coli, the isolation of linear DNA plasmids from Streptomyces, protoplast fusion of Brevibacterium flavum, protoplast fusion of various yeast genera, the introduction of isolated yeast mitochondria into Saccharomyces cerevisiae protoplasts, and the introduction of whole cells of the N-fixing bacteria Azotobacter vinelandii and Anacystis nidulans into Saccharomyces cerevisiae protoplasts.

=> s l1 and l3
L7 3 L1 AND L3

=> dup rem l7

PROCESSING COMPLETED FOR L7
L8 3 DUP REM L7 (0 DUPLICATES REMOVED)

=> s l8 not l6
L9 2 L8 NOT L6

=> d l9 ibib abs 1-2

L9 ANSWER 1 OF 2 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:748056 HCAPLUS
DOCUMENT NUMBER: 128:137042
TITLE: The Tsp45I restriction-modification system is plasmid-borne within its thermophilic host
AUTHOR(S): Wayne, Jay; Holden, Megan; Xu, Shuang-yong
CORPORATE SOURCE: New England Biolabs Inc., 32 Tozer Road, Beverly, MA 01915, USA
SOURCE: Gene (1997), 202(1/2), 83-88
CODEN: GENED6; ISSN: 0378-1119
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB ***Thermus*** species YS45 harbors two small cryptic plasmids of 5.8 (pTsp45s) and approx. 12 kb (pTsp45l). Plasmid pTsp45s has been entirely sequenced, revealing three significant ORFs. In addn. to a previously reported thermophilic plasmid-encoded replication protein (Rep), pTsp45s contains two genes for the Tsp45I methyltransferase (M.Tsp45I) and restriction endonuclease (Tsp45I). These two converging genes (tsp45IM and tsp45IR) overlap by 4 bp at their stop codons within an XbaI site. M.Tsp45I (413 aa, 47.0 kDa, recognizing 5'-GTSAC-3') is highly homologous to other m6A-methyltransferases, esp. M.EcaI (recognizing 5'-GGTNACC-3'). Tsp45I (332 aa, 37.4 kDa, cleaving 5'-.dwnarw.GTSAC-3') is not homologous to M.Tsp45I, or to other restriction endonucleases. Recombinant Tsp45I is stably produced in E. coli, and cleaves DNA at 65.degree.C with the same specificity as the native enzyme. Therefore, the thermophilic Tsp45I restriction-modification system is plasmid-borne within its native host.

L9 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:504779 HCAPLUS
DOCUMENT NUMBER: 127:230135
TITLE: Identification of a thermophilic plasmid origin and its cloning within a new ***Thermus*** -E. coli shuttle vector
AUTHOR(S): Wayne, Jay; Xu, Shuang-yong
CORPORATE SOURCE: New England Biolabs, 32 Tozer Road, Beverly, MA, 01915, USA
SOURCE: Gene (1997), 195(2), 321-328
CODEN: GENED6; ISSN: 0378-1119
PUBLISHER: Elsevier
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LANGUAGE: English
AB A pUC19-based vector has been generated for selecting functional thermophilic origins (oris) of ***Thermus*** ssp. Once combined with thermophilic DNA, the vector can be amplified in ampicillin resistant (ApR) E. coli, prior to transformation and kanamycin (Km) selection in ***Thermus*** thermophilus. The KmR ***Thermus*** transformants replicate any newly-formed shuttle vectors via introduced thermophilic oris. Using this 'ori-selecting' vector, three novel thermophilic oris were cloned from randomly digested ***Thermus*** cryptic plasmid DNA. These shuttle vectors are useful for genetic analyses, as well as protein engineering within thermophiles. The smallest ori-contg. sequence of 4.2kb has been subcloned, sequenced, and further refined to 2.3kb. A significant ORF of 341 amino acids (aa), with a ***Thermus*** promoter and RBS, is found within the thermophilic ori. Deleting part of this ORF abolishes the shuttle vector's ability to replicate in T. thermophilus. Therefore, we postulate that this ORF encodes a replication protein (Rep) necessary for thermophilic plasmid replication. The thermophilic ori also contains two sequences which resemble DnaA boxes.